Neurol Sci (2013) 34:1125–1131
DOI 10.1007/s10072-012-1190-y

ORIGINAL ARTICLE

The role of ethanol on the anticonvulsant effect of valproic acid and cortical microvascular changes after epileptogenesis in mice

Rahim Golmohammadi · Akbar Pejhan · Hassan Azhdari-Zarmehri · Mohammad Mohammad-Zadeh

Received: 17 July 2012 / Accepted: 3 September 2012 / Published online: 19 September 2012

Abstract There have been conflicting reports regarding the role of ethanol in seizure. Another effect of ethanol is vascular damage in cerebral tissue. This study investigates the influence of ethanol on antiepileptic efficacy of valproic acid (VPA) and cerebral microvascular structure. In this study, four groups of mice (25–30 g) received pentylenetetrazole (PTZ) i.p. (37 mg/kg) every other day. Different groups of animals received an injection of saline, ethanol (1 g/kg), VPA (100 mg/kg), or VPA and ethanol 30 min before PTZ. Animals in groups 5 and 6 received only ethanol and saline, respectively. After recording seizure parameters, the animals were sacrificed under deep anesthesia and the brains of the animals were removed and fixed, thereafter coronal sections were prepared from cerebral cortex. Then, the cerebral microvessels were counted in microscopic sections after hematoxylin–eosin staining. Ethanol injection (1 g/kg) for 7 days decreased stage 4 duration and increased latency to the onset of stage 1 and stage 4 of seizure ($p < 0.001$). Concomitant injection of VPA (5 min before ethanol) and ethanol had significantly stronger anticonvulsant effects than VPA alone ($p < 0.001$). Furthermore, the findings showed that not only the cerebral microvessels increased significantly in ethanol group compared with saline group ($p < 0.05$), but also there were morphological changes in vascular endothelium in ethanol group. The obtained results show that short-term ethanol administration has anticonvulsant effects along with VPA, and enhances the anticonvulsant effects of VPA. Furthermore, it is possible that VPA leads to decreased ethanol-induced vascular damage.

Keywords Seizure · Kindling · Valproic acid · Ethanol · Vascular damage

R. Golmohammadi
Department of Anatomy, Cellular and Molecular Research Center, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran

A. Pejhan · M. Mohammad-Zadeh (✉)
Department of Physiology, Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
e-mail: mohamad1353@gmail.com

H. Azhdari-Zarmehri
Cellular and Molecular Research Center,
Qazvin University of Medical Sciences, Qazvin, Iran

Introduction

Epilepsy is a common neurological disorder characterized by recurrent, unpredictable seizures with a prevalence rate of about 1 % [1]. A great deal of research is currently being conducted to reveal the mechanisms underlying epilepsy and also to find more effective drugs for epilepsy treatment. The available antiepileptic medications can be effective in the seizures in only about 40 % of cases, and they merely reduce the frequency of convulsions in other cases [2]. Laboratory models for epilepsy induction make it possible to analyze the mechanisms and predisposing factors of epilepsy and also to evaluate anticonvulsive drugs and treatment modalities. One of these models is the pentylenetetrazole (PTZ) kindling model [3, 4]. In this model of kindling, following repeated daily injections at the sub-threshold dosage of PTZ (37 mg/kg), seizure stages occur in succession [3].

Like many other diseases, risk factors should be considered for this neurological disorder. Alcohol consumption is one of the risk factors for this disease [5, 6]. Alcohol consumption is relatively common, particularly in industrialized countries. In 2002, about 40 % of 25- to 34-year-old Americans consumed alcohol at least five times per

month. The rate of consumption in younger population (18–24 years old) was about 51 % [7]. Another study showed that 77 % of men and 65 % of women in the USA reported alcohol consumption in 2001 [8].

There are contradictory reports about the effect of alcohol on epileptic seizures. Some studies reported an increase in frequency and severity of seizures in mice after alcohol consumption [9–11]. Conversely, other studies demonstrate that ethanol has an anticonvulsant effect [5, 12]. In most cases, this anticonvulsant effect occurs in short-term periods of alcohol consumption. There are also some studies reporting that alcohol had no effect on epileptic seizures. In non-alcoholic epileptic patients, low to moderate alcohol consumption had no effect on convulsions [6, 13, 14]. A wide range of fetal abnormalities, especially in the nervous system, have been reported to be caused by alcohol consumption [6, 15, 16]. Furthermore, it has been shown recently that alcohol consumption can impair the blood–brain barrier [17].

Valproic acid (sodium valproate; VPA) is one of the widely used antiepileptic drugs [18]. More recent studies report that VPA plays a role in inhibiting the death of progenitor cells producing neurons [19]. However, it is not perfectly clear whether VPA affects the cerebral microvascular structure in animals with PTZ-induced seizure receiving ethanol. In addition, in view of the fact that there are few reports regarding the effect of ethanol on the efficacy of antiepileptic drugs, this study was designed to investigate the impact of ethanol on the anticonvulsant effect of VPA and cerebral microvessel numbers after epileptogenesis in mice.

Methods

Animals

Forty male albino BALB/c mice (25–30 g), obtained from the animal house of Sabzevar University of Medical Sciences, were maintained in a colony room kept at a constant temperature on 12:12 light:dark schedule. The animals were individually housed in plastic cages with woodchip bedding and permitted free access to food and water. Procedures involving animals and their care were conducted in accordance with the "Guide to the Care and Use of Experimental Animals" [20]. All experiments were done at the same time (8:00 a.m. to 2:00 p.m.) in the morning to avoid the bias of circadian rhythms [21, 22].

Chemicals

Ethanol and sodium valproate were provided by Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from common commercial suppliers.

Chemical kindling in mice

Pentylenetetrazole was administered at a subconvulsant dose of 37 mg/kg on alternate days for a period of 24 days in control mice. PTZ injections were repeated 12 times. After each PTZ injection, the animal was placed in a chamber ($30 \times 40 \times 40$ cm) and their behavior was observed for 20 min. Convulsive responses to PTZ administration were classified as follows [3]: stage 0, no responses; stage 1, nodding or head clonus; stage 2, salivation and forelimb clonus; stage 3, spreading hind limbs and rearing on hind limbs; stage 4, falling backward or sideward with loss of righting reflex.

Experimental groups

Mice were divided into six groups. The control group (the first group) received vehicle (saline 0.9 %) 30 min before intraperitoneal PTZ; the second group was treated with ethanol (1 g/kg); the third group received VPA (100 mg/kg), and the fourth group received VPA and ethanol (1 g/kg). In these four groups, each experimental group had eight mice. The last groups (groups 5 and 6) received ethanol ($n = 4$) or saline ($n = 4$) alone. All animals, except the fifth and sixth groups, were injected with PTZ, 30 min after any intervention. Animals in group 2 (PTZ + ethanol) received ethanol for 7 days. Mice in group 3 received valproic acid along with 12 alternate-day injections of PTZ. In group 4, the animals received ethanol and VPA for 7 days (VPA 5 min before ethanol) and after that, they received only valproic acid 30 min before PTZ similar to group 3.

Sample preparation and histology

At the end of the experiment, the mice were deeply anesthetized with ether and perfused with saline and formalin 10 %. Their brains were removed and placed in 10 % formalin for at least 24 h at room temperature. The samples were fixed in formalin, dehydrated in a graded series of ethanol (30, 50, 70, 95, and 100 %), and treated with xylol for clearing. The slices from cortex were then embedded in paraffin. Sections of 5-μm thickness were prepared using a microtome (Leica Instruments, Germany) and stained with hematoxylin–eosin. Under a light microscope (Motic), 35 fields were selected using systematic random sampling for microvascular numbers counting. Microvessel counting was performed in a square measuring 0.64 mm^2. Microvessels were identified based on the absence of smooth muscle layer [23].

Statistical analysis

Data analysis was performed using SPSS statistical software package, version 11.5. Results were expressed as

mean ± standard error of the mean (SEM) and number of observations. One-way analysis of variance (ANOVA) and Dunnett test were used for statistical analysis. The basis of all decisions was a significance level of $p < 0.05$.

Results

Effect of ethanol injection on seizure parameters

During the 24-day period when PTZ was injected on alternate days, ethanol injection for 7 days decreased stage 4 duration (S_4D) of seizure significantly compared with PTZ + saline group ($F_{(11,44)} = 33.3$, $p < 0.001$) (Fig. 1). Ethanol injection (1 g/kg) for 7 days increased stage 4 latency (S_4L) significantly during 12 PTZ injections compared with PTZ + saline group ($F_{(11,99)} = 1.6$, $p < 0.05$) (Fig. 2). Furthermore, the results showed that ethanol injection (1 g/kg) for 7 days increased stage 1 latency (S_1L) significantly during 12 PTZ injections compared with PTZ + saline group ($F_{(11,154)} = 4.4$, $p < 0.001$).

Ethanol decreased mean seizure stage during 12 injections compared with PTZ + saline group. For this parameter, data are expressed in three points (at the third, seventh, and twelfth PTZ injections). Mean seizure stage in PTZ + saline group increased from 3.7 ± 0.3 (after the third PTZ injection) to 5 (after the twelfth PTZ injection). In PTZ + ethanol group, mean seizure stage decreased from 2.24 ± 0.4 (after the third PTZ injection) to 2.2 ± 0.5 (after the twelfth PTZ injection). The non-parametric Mann–Whitney U test showed that ethanol significantly decreased this parameter compared with PTZ + saline group after the third ($p < 0.05$), seventh ($p < 0.01$), and twelfth ($p < 0.01$) PTZ injections (Table 1).

Effect of VPA injection on seizure parameters

Compared with PTZ + saline group, VPA injection (PTZ + VPA group) caused a significant decrease (by 39 %) in S_4D ($F_{(9,45)} = 17$, $p < 0.001$), a significant increase (216.8 %) in S_4L ($F_{(9,81)} = 3.5$, $p < 0.001$), and also a significant increase in S_1L (149 %) ($F_{(9,126)} = 1.9$, $p < 0.05$) (Fig. 3). Mean seizure stage in PTZ + VPA group reached 1.8 ± 0.5 after the twelfth PTZ injection, showing a significant decrease compared with PTZ + saline group (5 ± 0) (Table 1).

Effect of VPA and ethanol injection on seizure parameters

The S_4D parameter decreased significantly in PTZ + VPA + ethanol group compared with PTZ + ethanol group (47 %) ($F_{(9,117)} = 9.2$, $p < 0.001$), as well as PTZ + VPA group (57.1 %) ($F_{(9,99)} = 6.5$, $p < 0.001$) (Fig. 1). In addition, the S_4L parameter in PTZ + VPA + ethanol group showed a 233.9 % increase compared with PTZ + VPA group ($F_{(9,99)} = 4.4$, $p < 0.001$) and a 450 % increase compared with PTZ + ethanol group ($F_{(9,126)} = 27$, $p < 0.001$) (Fig. 2).

In PTZ + VPA + ethanol group, mean seizure stage after the twelfth PTZ injection was 1.6 ± 0.4, showing no statistically significant difference when compared with PTZ + VPA group (1.8 ± 0.5) or PTZ + ethanol group (2.2 ± 0.5) (Table 1).

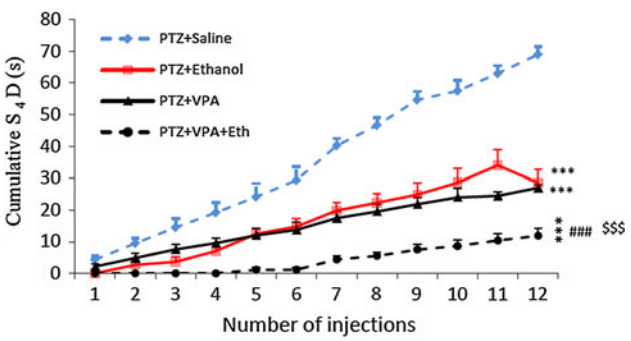

Fig. 1 Cumulative seizure stage 4 duration (cS_4D) in 12 PTZ injections. In PTZ + VPA + ethanol group, VPA was injected 5 min before ethanol. Ethanol injection for 7 days decreased cS_4D significantly. VPA injection decreased cS_4D compared with PTZ + saline group significantly, but it showed no significant difference with ethanol. Concomitant VPA and ethanol injection decreased cS_4D, but the difference with PTZ + VPA group was not statistically significant. ***$p < 0.001$ compared with PTZ + saline group. Data are expressed as mean ± SEM ($n = 8$)

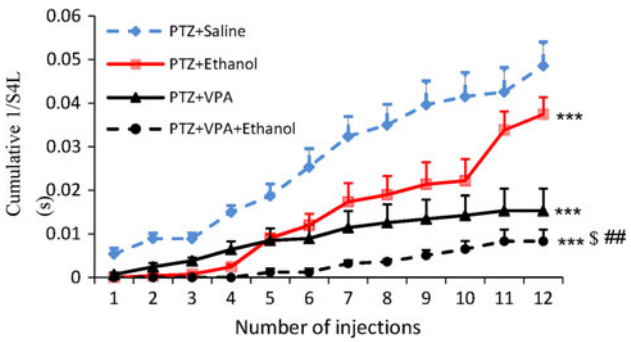

Fig. 2 Latency to the onset of stage 4 seizure (S_4L) (cumulative) in 12 PTZ injections shown as reverse value. In PTZ + VPA + ethanol group, VPA was injected 5 min before ethanol. Ethanol injection for 7 days decreased $1/S_4L$ significantly. Injection of VPA decreased $1/S_4L$ significantly compared with PTZ + saline group, but had no significant difference with ethanol group. Concomitant VPA and ethanol injection decreased $1/S_4L$ significantly compared with PTZ + VPA ($p = 0.03$) and PTZ + ethanol ($p = 0.003$) groups. ***$p < 0.001$ compared with PTZ + saline group. $^{\$}p < 0.05$ compared with PTZ + VPA group. $^{\#\#}p < 0.01$ compared with PTZ + ethanol group. Data are expressed as mean ± SEM ($n = 8$)

Table 1 Effect of ethanol, VPA and VPA + ethanol injection 30 min before PTZ application on the weekly average of seizure stage

Seizure stage	PTZ + saline	PTZ + ethanol	PTZ + VPA	PTZ + VPA + ethanol
After 3rd injection	3.7 ± 0.3	2.2 ± 0.4*	2.2 ± 0.5*	0.7 ± 0.3***,$,#
After 7th injection	4.1 ± 0.3	2.1 ± 0.3**	2 ± 0.5**	1 ± 0.3**
After 12th injection	5 ± 0	2.2 ± 0.5**	1.8 ± 0.5**	1.6 ± 0.4**

Data are expressed as mean ± SEM ($n = 8$)

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with PTZ + saline group, respectively

# $p < 0.05$ compared with PTZ + ethanol group

$ $p < 0.05$ compared with PTZ + VPA group

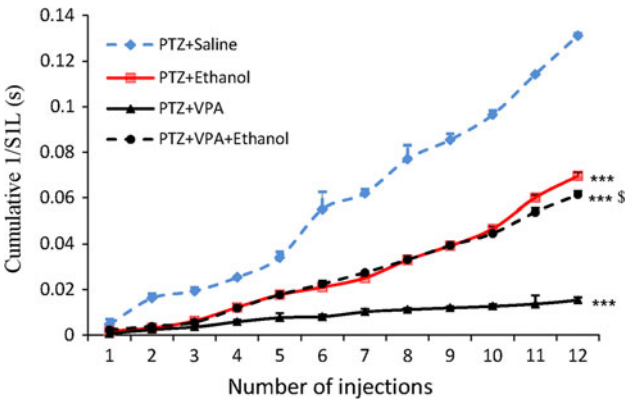

Fig. 3 Latency to the onset of stage 1 seizure (S_1L) (cumulative) in 12 PTZ injections shown as reverse value. In PTZ + VPA + ethanol group, VPA was injected 5 min before ethanol. Ethanol injection for 7 days decreased $1/S_1L$ significantly. Injection of VPA decreased $1/S_1L$ significantly compared with PTZ + saline group, but had no significant difference with ethanol group. Concomitant VPA and ethanol injection decreased $1/S_1L$ significantly compared with PTZ + saline group, but had no significant difference with PTZ + VPA or PTZ + ethanol group. ***$p < 0.001$ compared with PTZ + saline group. Data are expressed as mean ± SEM ($n = 8$)

Effect of ethanol and VPA on mean cerebral microvessel numbers

There was no difference in mean cerebral microvessel numbers between PTZ + saline (2 ± 0.96) and saline groups (1.7 ± 0.77). Mean cerebral microvessel numbers in PTZ + ethanol (2.4 ± 1.06) and PTZ + VPA (2.5 ± 1.03) groups were significantly different from PTZ + saline group ($p < 0.05$). On the other hand, mean cerebral microvessel numbers in ethanol group were increased (2.2 ± 0.96) relative to saline group (1.7 ± 0.77) ($p < 0.05$). Also, concomitant injection of ethanol and VPA in PTZ + VPA + ethanol group decreased cerebral microvessel numbers significantly relative to PTZ + VPA and PTZ + ethanol groups ($p < 0.5$) (Table 2).

Pathological findings of cerebral microvessels In PTZ + ethanol group, not only pathological changes, including thrombosis, and evident inflammatory reactions, but also considerable margination of blood cells was observed in endothelial walls of cerebral microvessels (Fig. 4e). In contrast, these changes were not observed in the PTZ + saline (Fig. 4a) and saline (Fig. 4b) groups.

Similarly, the animals in PTZ + VPA group showed no morphological changes, though the luminal diameter of cerebral blood vessels was larger in this group than the PTZ + saline group (Fig. 4c). In PTZ + VPA + ethanol group, vascular morphological changes and proliferation of endothelial cells were observed, but there was no vascular thrombosis (Fig. 4d).

Discussion

This study showed that ethanol injection (1 g/kg) for 7 days had anticonvulsant effects (it decreased S_4D and increased S_1L and S_4L). Concomitant injection of ethanol and VPA not only decreased PTZ-induced seizures considerably, but also inhibited the increase in microvessel

Table 2 Effect of ethanol, VPA and VPA + ethanol injection 30 min before PTZ injection on cerebral microvessel numbers

	Groups					
	PTZ + saline	PTZ + ethanol	PTZ + VPA	PTZ + VPA + ethanol	Ethanol	Saline
Microvessel numbers	2 ± 0.96	2.4 ± 1.06*	2.5 ± 1.03*	1.9 ± 1.33$	2.2 ± 0.96#	1.7 ± 0.77

Data are expressed as mean ± SEM

* $p < 0.05$ compared with PTZ + saline group

# $p < 0.05$ compared with saline group

$ $p < 0.05$ compared with PTZ + VPA and PTZ + ethanol groups

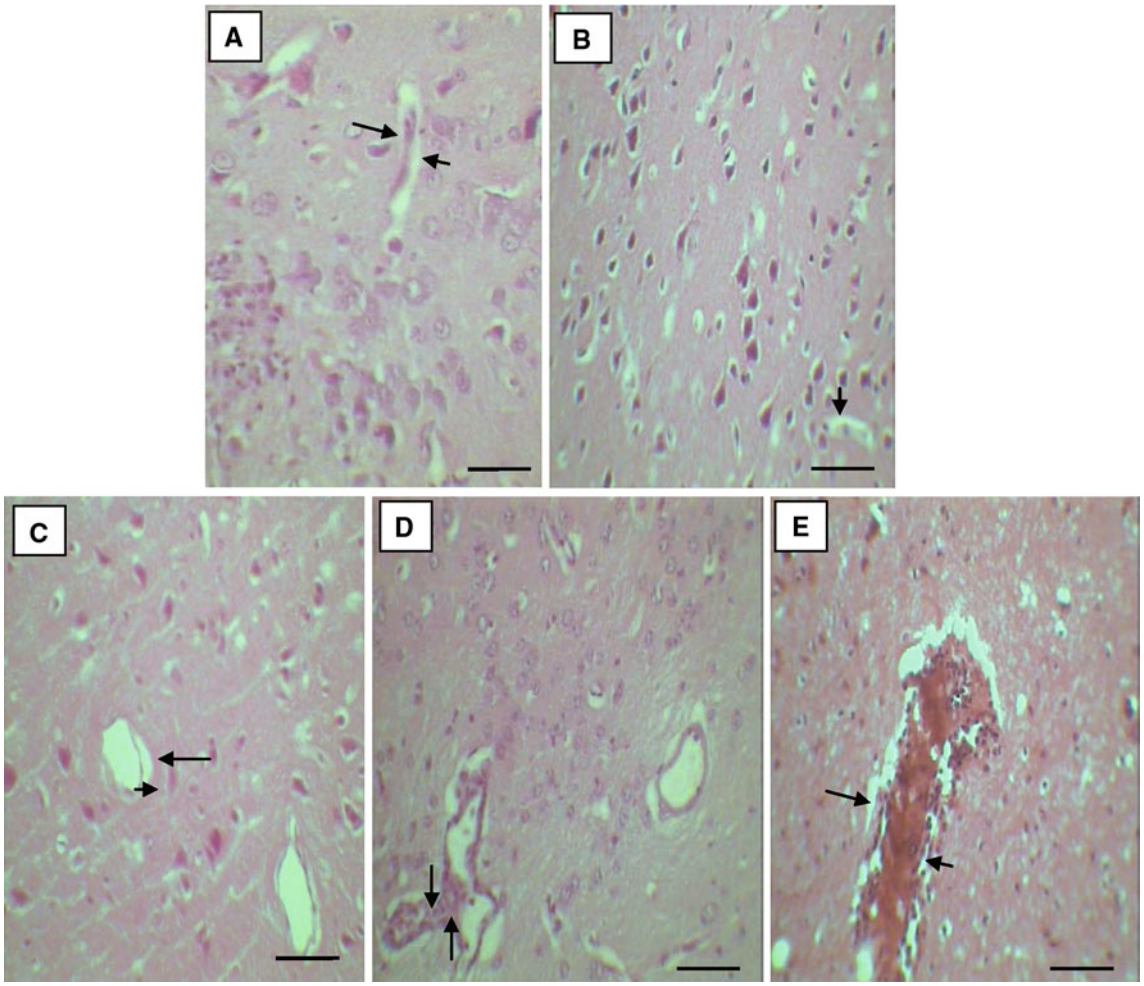

Fig. 4 Coronal 5-μm section of the cortex stained with hematoxylin–eosin in experimental groups. *Arrows* indicate endothelium and lumen of microvessels (**a**, **b** and **c**), proliferation of endothelial cells (**d**) and microvascular thrombosis (**e**). In PTZ + saline (**a**) and saline (**b**) groups, microvascular morphology was normal. In PTZ + VPA group (**c**), microvascular diameter was larger than PTZ + saline group. In PTZ + VPA + ethanol group (**d**), proliferation of endothelial cells was observed. In PTZ + ethanol group (E), thrombosis and margination of blood cells have been shown. Magnification ×400. *Scale bar* 50 μm

numbers caused by PTZ and ethanol alone and the vascular damage induced by PTZ.

The findings of this study are compatible with studies that report anticonvulsant effects for ethanol [9, 12, 24]. On the other hand, some studies have reported that ethanol is a proconvulsant. There are reports indicating that chronic alcohol consumption can increase the severity and frequency of convulsions [11, 25]. Also it has been shown that ethanol does not have any effect on seizures [6]. In fact, one reason for discrepancies in the effects of ethanol in various studies could be the difference in the number of daily injections and the type of epileptogenic agent used in those studies. The antiepileptic effect of VPA is thought to be due to the fact that VPA increases GABA levels in the brain and is able to block sodium channels [18]. In this way, VPA decreases the frequency and severity of seizure, since GABA is the most important inhibitory neurotransmitter in the brain [26]. On the other hand, one mechanism for the action of ethanol is through stimulation of GABA receptors [5]. Consequently, both compounds (VPA and ethanol) seem to act via the same pathway and so the inhibitory effect is stronger in PTZ + VPA + ethanol group. In accordance with these findings, it has been shown that concomitant injection of ethanol and VPA has no effect on blood concentration of VPA [5]. In other words, ethanol not only does not decrease VPA blood concentration, but also acts as an anticonvulsant and inhibits the progression of seizure to a greater extent than when taking VPA alone. In line with these results, it has been shown that in maximal electroshock-induced seizure and PTZ-induced seizure models, acute consumption of ethanol decreases ED50 of valproic acid, but chronic consumption of ethanol has no effect [27]. This means that acute consumption of ethanol enhances the anticonvulsant effect of VPA. Also in the

present study, the anticonvulsant effect of VPA was reinforced by the presence of ethanol.

In a study by Shiu et al. [28] on endothelial cells of human brain vessels, it was shown that ethanol causes vascular dysfunction and increases diffusion of blood components across endothelial cell membranes. In the current study, even though the animals receiving ethanol alone showed an increase in cerebral microvessel numbers, they had no indication of inflammatory reactions. On the other hand, PTZ + ethanol group showed both increased microvessels and inflammatory reactions. Actually, this finding shows that the blood–brain barrier is more vulnerable in the presence of both ethanol and PTZ. Since the structural changes of cerebral microvessels have a significant effect on neuronal function, these changes can lead to promotion or inhibition of epileptic seizures. For example, it has been shown that ischemia is a predisposing factor for development of seizures [18, 29]. On the other hand, some studies report an increase in angiogenesis during convulsions in epileptic patients [30]. It seems that decreased blood flow leads to excitation of neurons and development of seizures and the nervous system promotes angiogenesis during convulsions as a compensatory mechanism. The present study showed increased angiogenesis in PTZ + saline group, which can be justified by increased angiogenesis in epileptic subjects. Furthermore, PTZ + ethanol + VPA group showed no significant difference with saline or PTZ + saline group. It seems that this absence of changes is due to treatment and inhibition of seizures by both ethanol and VPA, thus preventing the induction of the compensatory mechanism of angiogenesis by the nervous system.

Some studies attribute ethanol-induced cellular damage to induction of enzymes activated in oxidative reactions, such as P450-2E1. Although the mechanism of action of alcohol on endothelial cells of cerebral vessels is not fully understood, it seems that increased stress reactions in vascular endothelial cells lead to dysfunction of the blood–brain barrier [30]. The fact that, in the present study, the effect of ethanol was stronger in PTZ + ethanol group than in the group receiving ethanol alone raises the question of whether ethanol acts synergistically with PTZ in damaging endothelial cells of cerebral vessels. More research is needed to answer this question, but it can be said that animals with PTZ-induced seizures are more susceptible to the effects of ethanol. Haorah and colleagues [31] showed that ethanol impairs IP_3 receptors, leading to release of calcium, which can cause neuronal death. In the present study, no inflammatory reaction was observed in PTZ + VPA + ethanol group. Therefore, it seems that VPA has been partially effective in repairing ethanol-induced damage to vascular endothelial cells. A report by Shang and colleagues [32] shows that VPA decreases cytokines involved in inflammatory reactions. This finding is compatible with the present study. Therefore, another contribution of this research is that VPA may partially prevent more vascular damage in patients with alcohol dependency. In accordance with this finding, it has been shown that VPA acts as a useful anticancer drug for treating patients with malignancy (VPA easily crosses the blood–brain barrier) [33].

In conclusion, according to the present results, it may be postulated that concomitant injection of ethanol and VPA has stronger anticonvulsant effects on PTZ-induced seizures. Also simultaneous use of ethanol and VPA inhibited the increase in microvessel numbers caused by seizure propagation and ethanol consumption alone, as well as the vascular damage induced by the epileptic disorder. Since VPA is an important antiepileptic agent and alcohol use is relatively prevalent, it is proposed that more cellular and molecular studies should be conducted to characterize the interaction effects of these drugs in epileptic disorders.

References

1. McNamara JO (1986) Kindling model of epilepsy. Adv Neurol 44:303–318
2. Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ (1999) New waves of research in the epilepsies: crossing into the third millennium. Adv Neurol 79:3–58
3. Barkai E, Grossman Y, Gutnick MJ (1994) Long-term changes in neocortical activity after chemical kindling with systemic pentylenetetrazole: an in vitro study. J Neurophysiol 72(1):72–83
4. Goddard GV, McIntyre DC, Leech CK (1969) A permanent change in brain function resulting from daily electrical stimulation. Exp Neurol 25(3):295–330
5. Fischer W (2005) Influence of ethanol on the threshold for electroshock-induced seizures and electrically-evoked hippocampal afterdischarges. J Neural Transm 112(9):1149–1163
6. Kozan R, Ayyildiz M, Yildirim M, Agar E (2006) The effects of ethanol intake and withdrawal on penicillin-induced epileptiform activity in rats. Brain Res Bull 71(1–3):111–115
7. Town M, Naimi TS, Mokdad AH, Brewer RD (2006) Health care access among US adults who drink alcohol excessively: missed opportunities for prevention. Prev Chronic Dis 3(2):A53
8. Fried VM, Prager K, MacKay AP, Xia H (2003) Health, United States: With Chartbook on Trends in the Health of Americans. National Center for Health Statistics, Hyattsville
9. Fischer W, Kittner H (1998) Influence of ethanol on the pentylenetetrazol-induced kindling in rats. J Neural Transm 105(10–12): 1129–1142
10. Hsieh CL, Chang CH, Chiang SY, Li TC, Tang NY, Pon CZ, Hsieh CT, Lin JG (2000) Anticonvulsive and free radical scavenging activities of vanillyl alcohol in ferric chloride-induced epileptic seizures in Sprague–Dawley rats. Life Sci 67(10):1185–1195
11. Scorza FA, Arida RM, Cysneiros RM, Priel MR, de Albuquerque M, Cavalheiro EA (2003) The effects of alcohol intake and withdrawal on the seizures frequency and hippocampal morphology in rats with epilepsy. Neurosci Res 47(3):323–328
12. Freeman FG (1978) Effects of alcohol on kindled seizure thresholds in rats. Pharmacol Biochem Behav 8(6):641–644

13. Hoppener RJ, Kuyer A, van der Lugt PJ (1983) Epilepsy and alcohol: the influence of social alcohol intake on seizures and treatment in epilepsy. Epilepsia 24(4):459–471
14. McQuarrie DG, Fingl E (1958) Effects of single doses and chronic administration of ethanol on experimental seizures in mice. J Pharmacol Exp Ther 124(3):264–271
15. Jaatinen P, Rintala J (2008) Mechanisms of ethanol-induced degeneration in the developing, mature, and aging cerebellum. Cerebellum (London, England) 7(3):332–347
16. Morleo M, Woolfall K, Dedman D, Mukherjee R, Bellis MA, Cook PA (2011) Under-reporting of foetal alcohol spectrum disorders: an analysis of hospital episode statistics. BMC Pediatr 11:14
17. Faria A, Pestana D, Teixeira D, Azevedo J, De Freitas V, Mateus N, Calhau C (2010) Flavonoid transport across RBE4 cells: a blood–brain barrier model. Cell Mol Biol Lett 15(2):234–241
18. Williams AJ, Tortella FC, Lu XM, Moreton JE, Hartings JA (2004) Antiepileptic drug treatment of nonconvulsive seizures induced by experimental focal brain ischemia. J Pharmacol Exp Ther 311(1):220–227
19. Go HS, Seo JE, Kim KC, Han SM, Kim P, Kang YS, Han SH, Shin CY, Ko KH (2011) Valproic acid inhibits neural progenitor cell death by activation of NF-kappaB signaling pathway and up-regulation of Bcl-XL. J Biomed Sci 18(1):48
20. Olfert ED, Cross BM, McWilliam AA (1993) Guide to the care and use of experimental animals, vol 1. Canadian Council on Animal Care, Ottawa
21. Yehuda S, Mostofsky DI (1993) Circadian effects of beta-endorphin, melatonin, DSIP, and amphetamine on pentylenetetrazol-induced seizures. Peptides 14(2):203–205
22. Pavlova MK, Shea SA, Bromfield EB (2004) Day/night patterns of focal seizures. Epilepsy Behav 5(1):44–49
23. Long MY, Li HH, Weng ZH (2008) Inhibitory effects of transfection of arresten gene on liver metastasis from colorectal cancer in nude mice. Chin J Cancer 27(10):312–315
24. Mladenovic D, Hrncic D, Vucevic D, Radosavljevic T, Loncar-Stevanovic H, Petrovic J, Susic V, Djuric D, Stanojlovic O (2007) Ethanol suppressed seizures in lindane-treated rats. Electroencephalographic and behavioral studies. J Physiol Pharmacol 58(4): 641–656
25. Silverstone PH, Williams R, McMahon L, Fleming R, Fogarty S (2008) Alcohol significantly lowers the seizure threshold in mice when co-administered with bupropion hydrochloride. Ann Gen Psychiatry 7:11
26. Olsen RW (2002) GABA. In: Davis KL et al (eds) Neuropsychopharmacology: the fifth generation of Progress Lippincott Williams & Wilkins, Philadelphia, pp 159–168
27. Kleinrok Z, Dziki M, Janczarek T (1993) The influence of ethanol on pentetrazol-induced seizures and anticonvulsant activity of phenobarbital and valproate against maximal electroshock in mice. Pol J Pharmacol 45(4):361–368
28. Shiu C, Barbier E, Di Cello F, Choi HJ, Stins M (2007) HIV-1 gp120 as well as alcohol affect blood–brain barrier permeability and stress fiber formation: involvement of reactive oxygen species. Alcohol Clin Exp Res 31(1):130–137
29. Uchino H, Smith ML, Bengzon J, Lundgren J, Siesjö BK (1996) Characteristics of postischemic seizures in hyperglycemic rats. J Neurol Sci 139(1):21–27
30. Rigau V, Morin M, Rousset MC, de Bock F, Lebrun A, Coubes P et al (2007) Angiogenesis is associated with blood–brain barrier permeability in temporal lobe epilepsy. Brain 130(Pt 7):1942–1956
31. Haorah J, Knipe B, Leibhart J, Ghorpade A, Persidsky Y (2005) Alcohol-induced oxidative stress in brain endothelial cells causes blood–brain barrier dysfunction. J Leukoc Biol 78(6):1223–1232
32. Shang Y, Jiang YX, Ding ZJ, Shen AL, Xu SP, Yuan SY, Yao SL (2010) Valproic acid attenuates the multiple-organ dysfunction in a rat model of septic shock. Chin Med J 123(19):2682–2687
33. Hrebackova J, Hrabeta J, Eckschlager T (2010) Valproic acid in the complex therapy of malignant tumors. Curr Drug Targets 11(3):361–379